High-throughput epitope identification for snakebite antivenom

Engmark, Mikael; De Masi, Federico; Laustsen, Andreas Hougaard; Gutiérrez, José María; Lomonte, Bruno; Andersen, Mikael Rørdam; Lund, Ole

Publication date: 2015

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

DTU Library
Technical Information Center of Denmark

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
High-throughput epitope identification for snakebite antivenom

Mikael Engmark1, Federico De Masi1, Andreas Hougaard Lausten2, José María Gutiérrez3, Bruno Lomonte3, Mikael Rordam Andersen1, and Ole Lund1

Introduction

Insight into the epitopic recognition pattern for polyclonal antivenoms is a strong tool for accurate prediction of antivenom cross-reactivity and provides a basis for design of novel antivenoms. In this work, a high-throughput approach was applied to characterize linear epitopes in 966 individual toxins from pit vipers (Crotalidae) using the ICP Crotalidae antivenom. Due to an abundance of snake venom metalloproteinases and phospholipase A2 in the venoms used for production of the investigated antivenom, this study focuses on these toxin families.

Epitopes locate to surface regions

To identify epitopes the observed peptide specific signal intensities were mapped back to the amino acid sequence of each pit viper toxin. Using two or more overlapping 15-mer peptides with median signals above 20 AU, epitope core sequences were localized and subsequently mapped to crystal structures or homology models. As examples, P-I metalloproteinase and lyso49-phospholipase A2 from Bothrops asper (venom used in antivenom production) are presented here.

Effect on cross-recognition

The m-helical shaped red epitope in the B. asper metalloproteinase is found to be highly conserved among pit viper metalloproteinases. Based on multiple sequence alignment of pit viper toxins sharing at least seven of the eight epitope residues and mean signal intensity of the eight 15-mer peptides harboring the epitope, we find that flanking residues outside of the core epitope has small effect on antivenom recognition. Expanding the analysis to the 42 toxins that share at least five of the epitope residues, residues outside of the core epitope has small effect on antivenom recognition.

Conclusions

- Custom-designed high density peptide microarray technology enables parallel automated identification of epitopes in hundreds of toxins.
- Integrating multiple sequence alignment allows investigation of the effect of epitope variation on antivenom recognition.
- Cross-reactivity of antivenom is correlated to the degree of conservation in toxin epitopes and flanking residues.

Acknowledgement

The peptide microarray experiments were performed at Schafer-N, Copenhagen. We would like to thank Claus Schafer, Christian Aukj Hansen, and Jens Krögel for experimental support and assistance. We further thank the Novo Nordisk Foundation for financial support (grant number: NNF13OC0005613).

References